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DNA, PROTAMINE AND METAL CONTENT OF INDIVIDUAL MAMMALIAN SPERM DETERMINED BY NUCLEAR MICROSCOPY.

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Pooled sample analyses have suggested that protamine defects in sperm nuclei and metal defects in semen correlate with male infertility. However, these analyses do not allow the examination of sperm sub-populations based on size or shape nor the variation of protamine or metals within the cell population. Quantitative single cell analyses can be used to supply such information and complement pooled sample data. We have used nuclear microscopy to measure the size, shape, mass, DNA content, protamine content and zinc content of individual fertile and infertile mammalian, male sperm nuclei. The technique produces average protamine and DNA masses per nucleus that agree well with available data from pooled sample analyses. Further, although the relative amounts of protamines 1 and 2 can vary widely in fertile semen of different mammalian species, we have found that the ratio of total protamine mass to DNA mass is remarkably similar for species in which little DNA remains packaged by histones. In addition, preliminary measurements suggest that protamine 2 may bind one zinc atom per protamine 2 molecule in normal fully functional mammalian sperm. Nuclear microscopy has also been used to study the expression of the protamine 2 gene in several lines of transgenic mice. This study has confirmed that mutant genes are being expressed and that extra protamine 2 (the amount of which we have estimated) is being deposited in the nucleus. Further, analyses of different sized epididymal sperm show that some protamine 2 present in late-step spermatids is lost during the final stages of maturation. These studies suggest that quantitative single cell analysis techniques such as nuclear microscopy may help resolve theories regarding the importance of protamines and metals to male fertility and help identify biochemical defects responsible for male infertility.